subsequent inoculation with the corresponding living organisms, a protection which, with appropriate doses of the endotoxin, is exerted for at least 11 weeks after the injection of the endotoxin. This result suggests that endotoxins may be of considerable value as protective or prophylactive vaccines. The endotoxin solutions maintain their activity for at least six weeks.

A few inoculations of typhoid and diphtheria endotoxins have been performed in the human subject. The inoculations cause some local reaction at the site of inoculation, consisting of redness, soreness and stiffness of the part, but little general reaction is induced, nor are any ill effects apparent.

I am indebted to Mr. Henry Wellcome for the facilities he has kindly afforded me for carrying out this work at the Wellcome Physiological Laboratories, and my best thanks are due to Mr. E. Thompson for his invaluable assistance in the preparation of the endotoxins.

On a Method of Disintegrating Bacterial and other Organic Cells.

By J. E. Barnard and R. T. Hewlett.

(Communicated by Prof. W. D. Halliburton, F.R.S. Received March 28,—Read May 4, 1911.)

[Plate 1.]

The methods hitherto available for accomplishing the disintegration of bacteria may be summarised briefly as follows:—The earliest experiment is that of Buchner* for obtaining the intracellular juices of yeast. This method was adopted by Macfadyen, Harris-Morris, and Rowland in their investigations on "Expressed Yeast Cell Plasma," communicated to the Royal Society, June 19, 1900, but they subsequently introduced some modifications and improvements in the method.

Their improved process was to place the yeast cells in mass in a mechanical contrivance, together with a proportion of added silver sand, and to violently agitate the containing vessel. The rapidly succeeding impacts of the yeast

* 'Berichte d. Deutsch. Chem. Ges.,' 1897, p. 117, and succeeding papers, 1897–1900.

cell with the sand particle ruptured the cell wall and caused the contents of the cell to be expelled.

The objection to this method is that a great rise of temperature rapidly takes place unless very efficient means are adopted for cooling; in fact, the whole mass may quickly reach boiling point unless this is efficiently performed. The cooling method they employed was to surround the containing vessel with brine at a temperature of about −5° C., which sufficed to keep the yeast mass at a temperature of about 15° C. Macfadyen and Rowland later discarded this method, and adopted one in which micro-organisms were disintegrated at the temperature of liquid air, and described their method in a paper on "The Intracellular Constituents of the Typhoid Bacillus."* In this apparatus the organisms in mass are placed in a cylindrical metal vessel, which is itself immersed in a vessel of liquid air. The inner container has a conical-shaped bottom, and in this another cone fits which is caused to rotate and is also free to move vertically. On placing the mass of bacterial or other cells in the container, the cells are rendered extremely brittle by the low temperature of the surrounding liquid air. The cone is caused to rotate inside the container and at the same time to move up and down, engaging at each rise and fall a number of the cells between the bottom of the containing pot and itself. The result is that each time a proportion of them is fractured. The sequence of operations is continued until the micro-organisms are found on microscopical examination to be disintegrated. On the completion of the process the temperature of the mass is allowed to rise, salt solution is added if necessary, and the suspension is then centrifugalised so that the cell bodies and any metallic contamination are removed. The amount that may be dealt with by this method is not large, varying usually from 0·5 to 1 grm., and the time required for the complete disintegration of such a quantity varies from one and a half to two hours.

Subsequent experiments have shown that while it is necessary to maintain the material at a low temperature to ensure the brittleness of the cell and to prevent chemical change during the process, such a low temperature as that of liquid air is not essential.

It appears to the writers that the conditions to be fulfilled in designing an efficient machine for the disintegration of micro-organisms are as follows:—

1. The grinding should be effected in a manner which is, as far as possible, frictionless, so that the risk of rise of temperature and consequent chemical change is avoided so far as possible, even apart from any extraneous cooling arrangement.

* 'Centralblatt für Bakteriologie,' 1903, No. 8.

2. Approximately, every micro-organism or cell should, sooner or later, be brought with certainty under the influence of the grinding action, so that the number of whole cells remaining is a minimum.

3. The containing vessel in which the grinding action takes place must be so effectually enclosed that during the process of disintegration no cells have any opportunity of escaping. This applies particularly to pathogenic organisms.

4. The appliance must be such that an efficient cooling arrangement may be adopted, and, if necessary, a temperature of 15° to 20° C. below freezing maintained at the actual point at which the grinding action takes place.

5. The action presumably requiring to go on in metallic containing vessels, it should be provided that the actual mechanical disintegration of metal between the grinding surfaces should be as little as possible.

These conditions are in the main complied with in the apparatus to be described (figs. 1 and 2).

The containing vessel consists of a phosphor-bronze body A, in which a number—usually five—of hardened steel balls, B, are placed. The shape of the containing vessel is such that when these balls are at its periphery they accurately fit the inner side of the vessel. The diameter of the vessel may conveniently be slightly less than the sum of the diameters of three balls. The balls are evenly distributed round the pot by means of a cage C, and, during the time they are running, this cage ensures that they are equidistant and do not collide one with another. At the centre of the metal pot is a steel cone D, which is of such a size that it keeps the balls in their proper position, in close contact with the periphery of the containing vessel. The vessel is closed by a screw cap E, through which the steel cone passes, and in which it is free to rotate. Over the whole of this a metal cylinder F is placed, and is screwed down, completely sealing the upper opening in the metal pot. In the top of this metal cylinder a steel bearing G is placed, which has freedom of movement in a vertical direction, but is kept down on the top of the steel cone by the action of a spring. It therefore follows that when this metal cylinder is screwed down, the steel cone is pressed down on to the balls, and the balls are in their turn forced out to the periphery of the metal pot. The whole appliance is mounted on a cone H, which is the upper end of a shaft passing through the base plate ; on the lower end of the shaft is a grooved wheel K, by which the apparatus may be rotated.

The grinding action is intended to take place between the steel balls contained in the metal pot and the interior surface of the pot, but it is evident that, so long as the whole appliance is rotated as it stands, no

grinding action would take place, as the vessel and its contents would rotate as a whole. To bring about a grinding or crushing action, a drag must be placed on the central steel cone, which retardation is in turn conveyed, at least in part, to the steel balls. It is therefore necessary

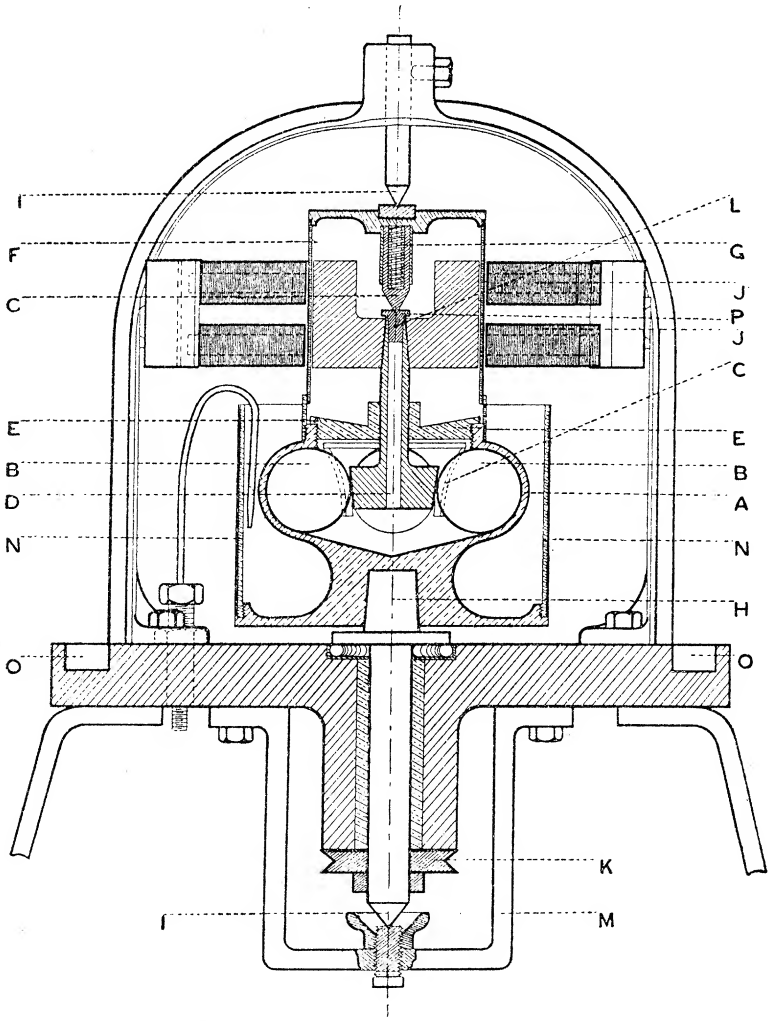


FIG. 1.—Apparatus for disintegrating bacterial or other cells. Type with electromagnetic control.

either to let the central steel cone remain at rest, or for it to rotate at a speed less than that of the steel balls. This has been effected in the type of machine now described (fig. 1), by mounting on the top end of the steel cone a bar of soft iron L, which is slightly less in length than the diameter of the covering cylinder. On each side of the machine, but

near to the outside of the covering cylinder F, a pair of electromagnets are mounted, with their poles in such a position that the iron bar on top of the steel cone is attracted by them. In actual practice it is found convenient and more efficient to let this electromagnet be a circular one, so that the magnetic circuit is closed, with a pole on each side, in such a position that it will attract the central iron armature. A suitable current of electricity is then passed through the winding of the electromagnets, so that the iron armature is kept in one position. It follows that, on rotating the containing vessel, while the armature is held by the electromagnets, a drag is put on the central steel cone, which in turn is communicated to the steel balls, and the grinding action occurs in the manner indicated.

To ensure that the bacteria are brought under the influence of the grinding

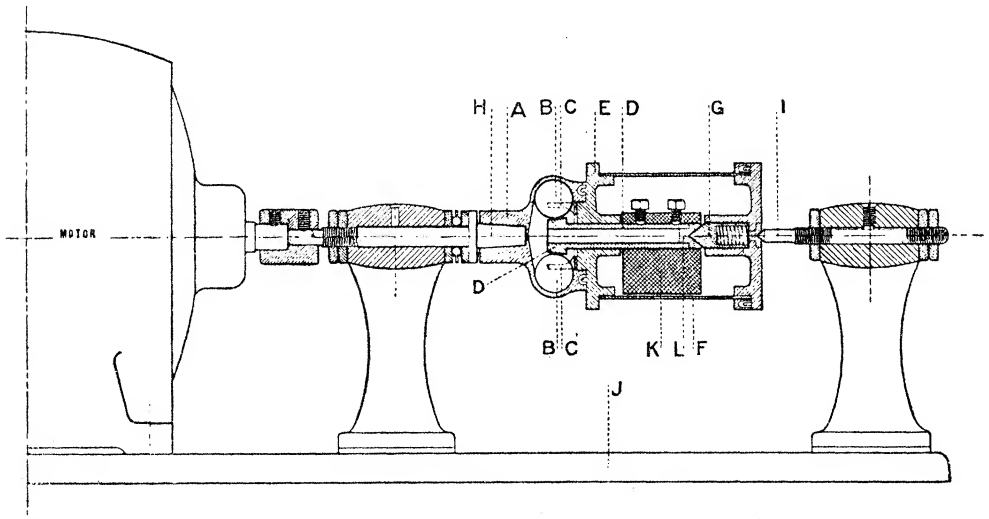


FIG. 2.—Apparatus for disintegrating bacterial or other cells. Type with gravitational control.

mechanism, the speed of rotation should be at least 1500 revolutions per minute. The bacteria are placed in the pot in a semi-fluid condition or as an emulsion. Centrifugal action then ensues, so that they are almost immediately brought under the grinding action of the balls. As the grinding action is one which takes place largely as the result of the rotation of the balls in an exactly fitting race the amount of friction is almost negligible; and further as the pressure that is put on the balls consists of a direct thrust by the steel cone, there is every opportunity for them to slip should any additional friction be introduced, or if for any reason any added load be put on the grinding mechanism.

To ensure that the running is perfectly true, the whole appliance is mounted between centres I, I, which are adjustable for wear.

Cooling may conveniently be effected by means of liquid carbonic acid. This may be obtained commercially, ready compressed in a steel cylinder, from which the gas may be allowed to escape and impinge on to the side of the metal pot. By varying the rate of escape of the gas the temperature may be controlled at will. An alternative method is to have another containing cylinder N outside the vessel in which the balls rotate, and to pack the space between this metal cylinder and the metal pot with a mixture of ice and salt or any other convenient freezing mixture.

If pathogenic organisms are being dealt with the whole machine may be covered with a glass bell-jar. Around the base-plate a groove O is cut, and in this any fluid bactericidal agent may be put, the glass jar being then placed over the whole, thus effectually preventing the possibility of the escape of any part of the contents of the pot.

The introduction of the material to be ground into the containing vessel is effected by unscrewing the top cylinder F and taking out the small plug P at the top of the steel cone. The spindle of the central steel cone is hollow, so that the emulsified bacteria may be introduced into the vessel by means of a pipette through this opening without disturbing the balls or any other part of the apparatus. On completion of the grinding action, owing to the cell contents of the bacteria having been expressed, the material is much more fluid than when introduced; and, therefore when the machine stops, it all sinks to the depressed centre of the containing vessel, and may be pipetted off again without further trouble. The introduction into, and removal of the material from, the metal pot without disturbance of any of the parts is a matter of considerable moment when dealing with pathogenic organisms. This arrangement also enables the ground material to be removed from the containing vessel immediately the grinding action ceases. As a further precaution the lid of the pot E is recessed towards its centre as shown: it is therefore possible to fill the hollow top of the lid with a bactericidal agent; immediately the machine is started the speed of rotation ensures that this fluid is thrown outwards by centrifugal action, and completely seals the two screw-joints where the lid of the pot and the outer cylindrical cover meet. As a point in the construction it is necessary that the side wall of the top cylindrical cover should be made of vulcanite or some diamagnetic material, as otherwise the necessary magnetic pull on the armature inside would not be effected.

A simplified form of the appliance is one in which the whole apparatus is mounted horizontally, and the pull is put on the central steel cone not through the agency of an electromagnet, but by means of gravity (fig. 2). The whole arrangement is exactly similar to that previously described,

except that the pot, with its balls, steel cone, and outer cover, is mounted horizontally between centres.

On the spindle of the central steel cone D a semi-cylindrical mass of iron or lead K is fixed, the weight of which has to be calculated for and proportioned to the drag on the central cone, but must be such that when the whole apparatus is rotated the weight is sufficient to keep the central cone at rest. In practice this modification has proved to be simpler than the original model, the only objection being that it is not quite so easy to cover the whole apparatus with an outside bell-jar as in the first described type.

The following experiments were carried out with the apparatus described :—

YEAST.

In order to ascertain the efficiency of the grinding under different conditions, a series of experiments was performed with yeast. The yeast cells being large, and their cell membranes being readily recognisable after the cells have been crushed, it was possible to make an actual count of the crushed and uncrushed cells after grinding. Fresh German yeast was used and this was made into a smooth stiff cream with water for the purpose of grinding. Films were made on glass slides at various stages of the grinds; these were stained with Löffler's methylene blue, and the counts made. Controls of the unground yeast showed that practically all the cells were intact.

The results obtained were as follows :—

I. Amount = 6 c.c. yeast cream. Speed = 700—750 revolutions per minute.

(*a*) After 20 minutes' grind. Crushed cells = 597, uncrushed cells = 87. Percentage of crushed cells = 87.

(*b*) The same ground for a further period of 20 minutes. Crushed cells = 646, uncrushed cells = 32. Percentage of crushed cells = 96.

II. Similar to I. Total period of grind = 20 minutes. Examined at intervals of 5 minutes.

(*a*) After 5 minutes' grind. Crushed cells = 63, uncrushed cells = 226. Percentage of crushed cells = 21·4.

(*b*) After 10 minutes' grind. Crushed cells = 284, uncrushed cells = 343. Percentage of crushed cells = 45.

(*c*) After 15 minutes' grind. Crushed cells = 412, uncrushed cells = 113. Percentage of crushed cells = 78.

(*d*) After 20 minutes' grind. Crushed cells = 694, uncrushed cells = 59. Percentage of crushed cells = 92.

III. Amount = 12 c.c. of yeast cream. Speed 700—750 revolutions per minute. Total period of grind = 30 minutes. Examined at intervals of 10 minutes.

(*a*) After 10 minutes' grind. Crushed cells = 380, uncrushed cells = 326. Percentage of crushed cells = 53·8.

(*b*) After 20 minutes' grind. Crushed cells = 297, uncrushed cells = 195. Percentage of crushed cells = 60.

(*c*) After 30 minutes' grind. Crushed cells = 473, uncrushed cells = 109. Percentage of crushed cells = 81.

IV. Amount = 3 c.c. of yeast cream. Speed 700—750 revolutions per minute.

(*a*) After 10 minutes' grind. Crushed cells = 216, uncrushed cells = 54. Percentage of crushed cells = 80.

(*b*) After 20 minutes' grind. Crushed cells = 192, uncrushed cells = 32. Percentage of crushed cells = 85.

(*c*) After 30 minutes' grind. Crushed cells = 210, uncrushed cells = 40. Percentage of crushed cells = 84.

V. Amount = 18 c.c. of yeast cream. Speed 700—750 revolutions per minute.

(*a*) After 10 minutes' grind. Crushed cells = 46, uncrushed cells = 122. Percentage of crushed cells = 27.

(*b*) After 20 minutes' grind. Crushed cells = 127, uncrushed cells = 98. Percentage of crushed cells = 56.

(*c*) After 30 minutes' grind. Crushed cells = 154, uncrushed cells = 65. Percentage of crushed cells = 77.

VI. Amount = 6 c.c. of yeast cream. Speed 500 revolutions per minute.

After 20 minutes' grind. Crushed cells = 219, uncrushed cells = 53. Percentage of crushed cells = 80·5.

VII. Amount = 12 c.c. of yeast cream. Speed about 500 revolutions per minute.

After 20 minutes' grind. Crushed cells = 178, uncrushed cells = 79. Percentage of crushed cells = 69.

VIII. Amount = 12 c.c. of yeast cream. Speed 2000 revolutions per minute.

After 20 minutes' grind. Crushed cells = 141, uncrushed cells = 59. Percentage of crushed cells = 70·5.

IX. Amount = 12 c.c. of yeast cream. Speed 360 revolutions per minute.

After 20 minutes' grind. Crushed cells = 125, uncrushed cells = 189. Percentage of crushed cells = 39·5.

The machine did not run well at this low speed.

X. Amount = 12 c.c. of yeast cream. Speed 1400 revolutions per minute.

After 20 minutes' grind. Crushed cells = 134, uncrushed cells = 75. Percentage of crushed cells = 64.

XI. Amount = 6 c.c. of yeast cream. Speed 828 revolutions per minute.

After 20 minutes' grind. Crushed cells = 85, uncrushed cells = 18. Percentage of crushed cells = 82·5.

XII. Amount = 18 c.c. of yeast cream. Speed 750—800 revolutions per minute.

After 20 minutes' grind. Crushed cells = 233, uncrushed cells = 100. Percentage of crushed cells = 70.

XIII. Amount = 6 c.c. of yeast cream. Speed 700—750 revolutions per minute.

After 20 minutes' grind. Crushed cells = 188, uncrushed cells = 68. Percentage of crushed cells 73·5.

In Plate 1, *a* is a photo-micrograph of unground yeast, *b* and *c* the same after grinding for 15 and 30 minutes respectively.

BACTERIA.

A direct microscopical count in the case of bacteria is impossible, and the results of grinding were therefore estimated—

(1) By a general microscopical survey of the specimens,

(2) By plating and ascertaining the number of surviving micro-organisms.

I. Cream of *Bacillus coli*. Amount = 3 c.c.

(*a*) Speed 700 revolutions per minute. Result: a good grind in 1 hour.

(*b*) Speed 1400 revolutions per minute. Result: almost complete grind in 1 hr.

(*c*) Speed 2400 revolutions per minute. Result: in $\frac{1}{4}$ hour, good grind—nearly as good as in 1 hour at 700 revolutions; in $\frac{1}{2}$ hour almost complete grind.

II. Cream of *B. coli*. Amount = 3 c.c. Speed 1700 revolutions per minute. Number of living organisms estimated by means of agar plates.

Colonies on the Plates.

Controls before grinding.	After grinding.			
	15 minutes.	30 minutes.	45 minutes.	60 minutes.
1/100,000 dil. 0·1 c.c. 405 colonies	1/100,000 dil. 0·1 c.c. 77 colonies	1/10,000 dil. 0·1 c.c. 138 colonies	1/10,000 dil. 0·1 c.c. 134 colonies	1/1000 dil. 0·1 c.c. 86 colonies
1/100,000 dil. 0·5 c.c. 1978 colonies	1/100,000 dil. 0·5 c.c. 342 colonies	1/10,000 dil. 0·5 c.c. 678 colonies	1/10,000 dil. 0·5 c.c. 468 colonies	1/1000 dil. 0·5 c.c. 314 colonies
1/100,000 dil. 1·0 c.c. uncountable	1/100,000 dil. 1·0 c.c. 576 colonies	1/10,000 dil. 1·0 c.c. 1280 colonies	1/10,000 dil. 1·0 c.c. 1024 colonies	1/1000 dil. 1·0 c.c. 640 colonies

Average per Unit Volume.

Control before grinding.	After grinding.			
	15 minutes.	30 minutes.	45 minutes.	60 minutes.
400,300,000	67,700,000	13,053,000	11,000,000	709,300

With other bacilli, *e.g.*, *B. megaterium*, *B. mycoides*, *B. subtilis*, and *B. typhosus*, equally good results were obtained. In Plate 1, *d* is a photo-

micrograph of unground *B. mycoides*, *e* and *f* the same after grinding for 15 and 30 minutes respectively.

III. Cream of *Micrococcus pyogenes aureus*. Amount = 3 c.c. Speed 1700 revolutions per minute. Number of living organisms estimated by means of agar plates.

Colonies on the Plates.

Controls before grinding.	After grinding.			
	15 minutes.	30 minutes.	45 minutes.	60 minutes.
1/1,000,000 dil. 0·1 c.c. 2 colonies	1/1,000,000 dil. 0·1 c.c. 1 colony	1/100,000 dil. 0·1 c.c. 13 colonies	1/100,000 dil. 0·1 c.c. 14 colonies	1/10,000 dil. 0·1 c.c. nil
1/1,000,000 dil. 0·5 c.c. 42 colonies	1/1,000,000 dil. 0·5 c.c. 12 colonies	1/100,000 dil. 0·5 c.c. 33 colonies	1/100,000 dil. 0·5 c.c. 27 colonies	1/10,000 dil. 0·5 c.c. 14 colonies
1/1,000,000 dil. 1·0 c.c. 145 colonies	1/1,000,000 dil. 1·0 c.c. 72 colonies	1/100,000 dil. 1·0 c.c. 41 colonies	1/100,000 dil. 1·0 c.c. 24 colonies	1/10,000 dil. 1·0 c.c. 27 colonies

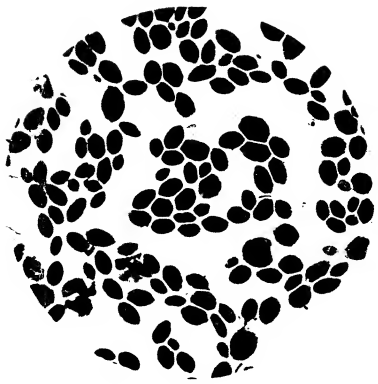
Average per Unit Volume.

Control before grinding.	After grinding.			
	15 minutes.	30 minutes.	45 minutes.	60 minutes.
83,000,000	35,300,000	7,900,000	7,270,000	275,000

In Plate 1, *g* is a photomicrograph of unground *M. pyogenes aureus*, *h* and *i* the same after grinding for 15 and 30 minutes respectively.

Conclusion.

We believe that the results of these experiments show that the apparatus here described does efficiently disintegrate bacterial cells. The apparatus is simple to manipulate, and, moreover, its design provides absolutely against the escape of any of the contents in the process of grinding, a consideration of great moment when dealing with pathogenic micro-organisms.

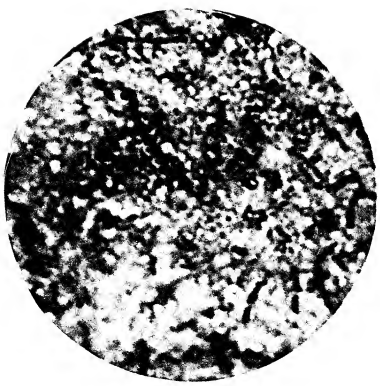


A

B

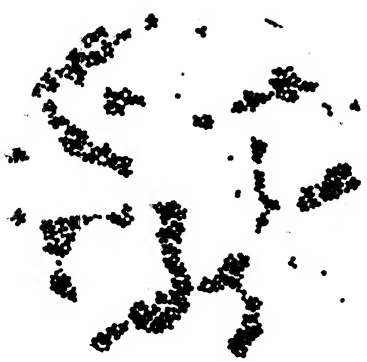
C

D

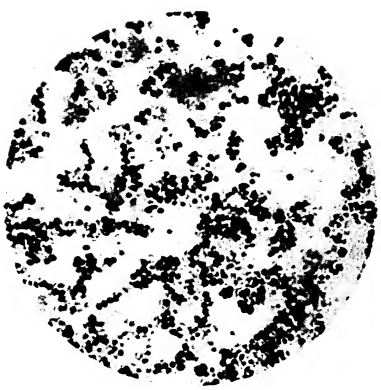


E

F



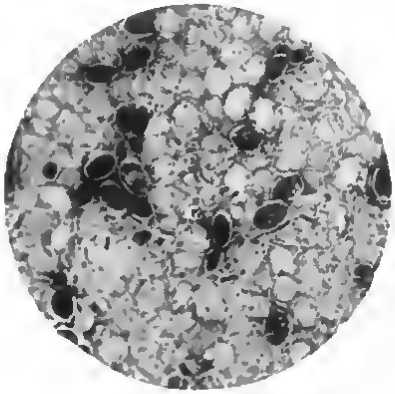
G



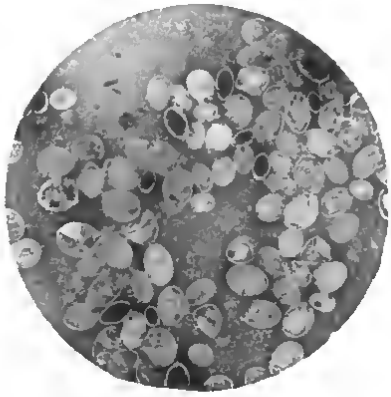
H

I

A



B



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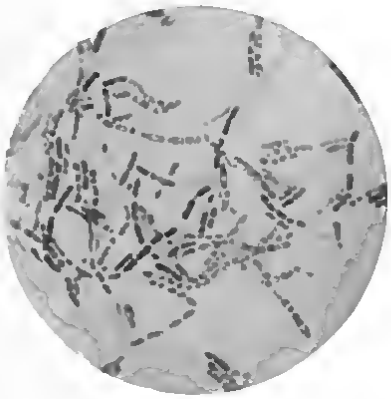
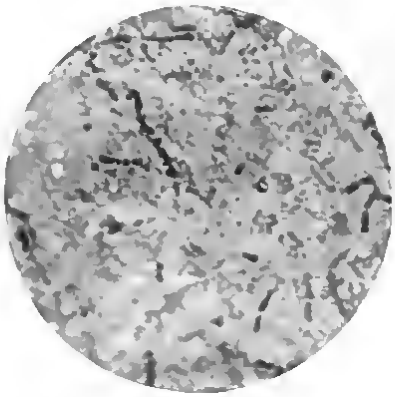
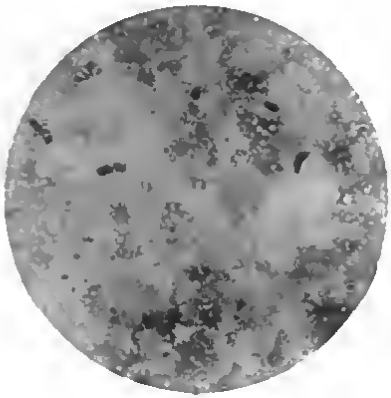


D



E



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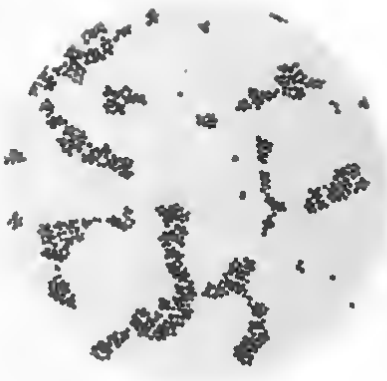
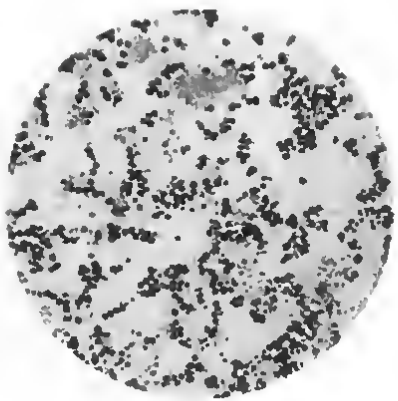


G



H

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